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Effects of seminal plasma and different cryoprotectants on rabbit sperm preservation at 16°C

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Abstract: The purpose of this research was to assess whether the presence of seminal plasma (SP) can improve sperm quality of rabbit spermatozoa stored at 16°C for 72 h and moreover evaluate the cryoprotectant effects of glycerol, N-N-Dimethylformamide (DMF), and N-methyl-2-pyrrolidone (NMP). Semen samples were pooled and divided in eight fractions. Four of them were diluted with INRA (extender A), INRA with 6% glycerol (extender B), INRA with 6% DMF (extender C), or INRA with 6% NMP (extender D), respectively. The other four fractions were centrifuged, and the supernatant was removed in order to eliminate SP. Each sample was then resuspended with extender A, B, C, or D, respectively. All samples were stored at 16°C and analysed at 4, 24, 48, and 72 h by ISAS®, vitality test, HOS test, and acrosome integrity test. After analyse of the results, SP samples showed a significantly higher percentage *(P*=0.020*)* in the HOS test (71.9 ± 1.6%) than non-SP samples (66.5 ± 1.6%). Non-SP samples had better results for kinematic parameters. Extenders A and C showed great results for the percentage of motile spermatozoa (63.1 ± 4.3% and 63.4 ± 3.7%, respectively), vitality (88.9 ± 2.6% and 87.7 ± 2.7%, respectively), and HOS test (68.9 ± 1.4% and 75.2 ± 1.4%, respectively). Extenders B and D showed worse data for sperm quality. These results suggest that SP has a protective effect on rabbit sperm membranes and maintains better sperm motility. The addition of glycerol and NMP to INRA does not improve rabbit sperm quality; nevertheless, the DMF cryoprotectant exerts a protective effect on the membrane of spermatozoa, improving seminal quality during rabbit sperm preservation at 16°C.

Key words: dimethylformamide, glycerol, N-methyl-2-pyrrolidone, seminal plasma, sperm preservation

Introduction

Seminal plasma (SP) is the liquid fraction of semen which is involved in sperm survival and competence, embryo development, endometrial receptivity, and ovarian function in females after insemination [4, 22, 44, 45]. The constituents of SP and its functions are not completely clear in rabbits due to the lack of studies. To date fructose, sorbitol, citric acid, inositol, glycerol, ergothioneine, glutamic acid, electrolytes, glycerylphosphor-

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ylcholine, proteins such as ovulation-inducing factor (OIF) [42], glycoproteins, enzymes, and minerals such as sodium, potassium, phosphorus, magnesium, calcium, and zinc are known to be included in SP [22, 41]. Each SP constituent has a different role; thus OIF is one of the main proteins which stimulates luteinizing hormone secretion, ovulation, and luteal gland development of induced and spontaneous ovulation species [1]. Additionally, OIF increases the total number of antral follicles and haemorrhagic anovulatory follicles in rabbits [42]. Conversely, SP presents a glycoprotein which reversibly inhibits the sperm capacitation process and acrosome reaction [29].

Previous studies have shown the deleterious effect of SP on preservation of bovine [18, 40], ovine [18], and equine sperm [6, 33]. In contrast, SP showed beneficial effects on buffalo [40] and Iberian red deer semen [31]. In rabbits, the presence of SP has shown a slight beneficial effect on sperm quality [2, 10, 17].

As is well known, storage temperature and the extender play an important role in spermatozoon survival [9]. Temperatures of 5°C to 25°C are suitable for rabbit sperm preservation for 72 h [25, 28, 39]. Currently, commercial artificial insemination centres carry seminal doses to other farms at a chilled temperature of around 16°C.

The characteristics of rabbit sperm (high activation energy and low water permeability coefficient) must be considered in relation to the cryoprotectants used [13, 32]. Therefore amide or methyl group cryoprotectants, which are characterised as having lower molecular weights and higher permeability, may be appropriate to use in rabbit sperm preservation [14].

N-N-Dimethylformamide (DMF) is an amide solvent that could be used to preserve mammalian sperm. Previous studies demonstrated that DMF is a better cryoprotectant than glycerol for stallion sperm [36, 37]. Conversely, it has been shown in the boar [30], dog [15, 27, 34], goat [7], and fowl [12] that sperm quality gets worse when DMF is used instead as extender for glycerol. Another amide solvent with similar characteristics to DMF is N-methyl-2-pyrrolidone (NMP). NMP presents an advantage compared with DMF: a carcinogenic nature has not been demonstrated for it to date [20].

The few studies carried out on SP and glycerol effect on rabbit sperm preservation and the absence of studies on preserving rabbit sperm with DMF or NMP as the cryoprotectant led us to investigate further the effects of SP and these three cryoprotectants. In addition, the main importance of using rabbit sperm as a laboratory model is due to its similarity to human spermatozoa, though here are also many other advantages, such as the size of the animals themselves (small animals are easy to handle), simple manner in with to obtain samples (unlike the mouse, it is not necessary to sacrifice the animal to get sperm samples), quicker means of obtaining results (a doe's pregnancy lasts around l month), and the ability to perform the same experiment in other species if the results are suitable. Therefore, the purpose of this research was to determine whether there is any difference in quality of rabbit sperm stored at 16°C among samples stored with SP or without SP. Furthermore, different cryoprotectants were tested in order to determine if they have any beneficial effects on semen quality of rabbit sperm stored at 16°C for 72 h.

Materials and Methods

Chemicals

Unless otherwise stated, all the chemicals were from Panreac Quimica S.L.U. (Barcelona, Spain).

Animals

The study was performed following approval by the Veterinary Ethics Committee of the University of Zaragoza. The care and use of animals were performed according to the Spanish Policy for Animal Protection (RD1201/05), which meets the European Union Directive 86/609 on the protection of animals used for experimental and other scientific purposes.

The rabbits were housed with a controlled light cycle of 12 h light/dark, room temperature of 22–24°C, and 55–60% relative humidity in a commercial artificial insemination center (Técnicas Cunícolas S.A.). They were fed a commercial pellet diet according to their reproductive needs, and fresh water was provided *ad libitum*. Eight mature rabbits were tested previously and selected for this research.

Semen collection and processing

Semen was collected using an artificial vagina (IMV Technologies, L'Aigle, France), and any gel plugs were removed. Before dilution, macroscopic analyses of each ejaculate were performed for assessment of the colour and the volume of the sample; the first microscopic analyses of motility were also performed. Only ejacu-

lates that were white in colour, had a volume >0.2 ml, and had at least 85% motility were used for the study.

All ejaculates were pooled in order to eliminate individual differences, and afterwards heterospermic samples were divided into eight fractions. Four of them were diluted (1:5) with four different semen extenders: INRA 96® (IMV Technologies, L'Aigle, France) (extender A), INRA 96® supplemented with 6% glycerol (extender B), INRA 96® supplemented with 6% DMF (extender C), and INRA 96® supplemented with 6% NMP (extender D). The other four fractions were centrifuged once at 700 × g for 10 min, and the supernatant was removed in order to eliminate the maximum SP. Then each pellet was resuspended with extender A, extender B, extender C, or extender D.

Subsequently, all samples were cooled progressively from 37°C to 16°C over the course of 120 min [32] and stored at 16°C for 72 h.

Evaluation of spermatozoa

Sperm samples were assessed at 4, 24, 48, and 72 h after collection for the different extenders with and without SP. The following tests were performed.

Sperm motility and kinematics: Sperm motility and kinematics were analysed by ISAS® software (PROISER R+D, Valencia, Spain) with the default setting specifically for rabbits. The following parameters were assessed: percentage of motile spermatozoa (MOT;%), curvilinear velocity (VCL; μm/s), straight-line velocity (VSL; μm/s), average path velocity (VAP; μm/s), linearity (LIN=VSL/VCL;%), straightness (STR=VSL/VAP;%), wobble (WOB=VAP/VCL;%), amplitude of lateral head displacement (ALH; μm), and beat cross frequency (BCF; Hz).

Vitality: The vitality of spermatozoa was evaluated by eosin-nigrosin staining. An aliquot of semen was put on a glass slide and mixed with an equal volume of eosin-nigrosin suspension, and a smear was then made on a glass slide. Immediately after the smear was dry, 100 spermatozoa in each replicate were examined by microscope at ×400 magnification and counted with the aid of a laboratory counter [8, 46]. Live spermatozoa had an undamaged membrane that could not penetrated by the colouring agent, resulting in spermatozoa with white heads (unstained). On the other hand, dead spermatozoa had red or dark pink heads (stained) due to damage to the membrane that allowed the colouring agent to penetrate into the cell.

Sperm plasma membrane integrity: The hypo-osmotic swelling test (HOS test) was based on the resistance of the semi-permeable membrane when it was subjected to a hypo-osmotic medium [24]. In the test, 90 μl of HOS test solution (100 mM of sodium citrate) was placed into an Eppendorf tube to warm at 37°C for 5 min. Once it was heated, it was added to 10 μl of semen, and the mixture was mixed gently with a pipette and kept at 37°C for at least 30 min. Subsequently, 100 μl of 2% glutaraldehyde was added to fix the sample. Ten microliters of the mixture was placed onto a glass slide and covered with a coverslip for examination of 100 spermatozoa in each replicate by phase-contrast microscope at ×400 magnification [5, 46]. Therefore, spermatozoa with intact membranes allowed an influx of water inside them resulting in swollen spermatozoa with coiled tail.

Acrosome integrity: The acrosome integrity test is used to determine the morphology of the acrosome and whether it is damaged. Ten microliters of semen was immediately fixed in 90 μl of glutaraldehyde 2% solution, and then 10 μl of the mixture was immediately placed onto a glass slide and covered with a coverslip for examination of 100 spermatozoa in each replicate by phase-contrast microscope at ×1,000 magnification with immersion oil [38].

Statistical analysis

The study was replicated three times. Data were analysed using IBM SPSS Statistics 19.0 for Windows. Results were expressed as the mean ± SEM. The values concerning the effects of the extenders, SP, and durations of storage were analysed as fixed factors in a full factorial General Linear Model (GLM), since we included all possible combinations of extenders, presence/absence of SP, and durations of storage. Duncan's post hoc test was used to evaluate the effects of the extenders and durations of storage. The interactions among the diluent used, SP presence, and storage duration were analysed using the GLM procedure. The level of significance was set at $P<0.050$.

Results

The data obtained after analysis of motility, kinematic parameters, vitality, and membrane damage of cool rabbit sperm treated with or without SP are synthesised in Table 1 and Fig. 1. The effects of SP on the LIN, STR and WOB parameters and HOS test were statistically

Table 1. Effect of seminal plasma on kinematic parameters of rabbit spermatozoa from the four extender groups (A–D)

	MOT (%)	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)	LIN (%)	STR (%)	WOB (%)	ALH (μm)	BCF (Hz)
With SP	52.4 ± 2.7	69.3 ± 2.8	21.9 ± 1.3	36.2 ± 1.6	32.2 ± 1.2	60.1 ± 1.1	53.0 ± 1.2	3.3 ± 0.1	7.7 ± 0.3
Without SP	47.1 ± 2.9	69.8 ± 2.9	24.5 ± 1.4	38.1 ± 1.7	36.3 ± 1.3	62.8 ± 1.1	56.2 ± 1.2	3.1 ± 0.1	7.0 ± 0.3
P	0.150	0.890	0.150	0.435	0.007	0.041	0.023	0.410	0.094

Data are shown as the mean ± SEM.

Table 2. Effect of extender A–D on kinematic parameters of rabbit spermatozoa from the with and without SP groups

	MOT (%)	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)	LIN (%)	STR (%)	WOB (%)	ALH (μm)	BCF (Hz)
Extender A	63.1 ± 4.3^{a}	76.2 ± 4.3^{ab}	32.9 ± 2.0^{a}	49.1 ± 2.5^{a}	41.2 ± 1.9^{a}	64.7 ± 1.7^{a}	62.1 ± 1.8^{a}	2.8 ± 0.2^{b}	7.7 ± 0.4^{a}
Extender B	28.6 ± 4.3^{c}	63.6 ± 4.3^{bc}	21.2 ± 2.0^{b}	34.1 ± 2.5^{b}	36.2 ± 1.8^{b}	62.1 ± 1.7^{a}	56.8 ± 1.8^{b}	3.0 ± 0.2^{b}	6.7 ± 0.4^{a}
Extender C	63.4 ± 3.7^{a}	81.3 ± 3.8^{a}	22.0 ± 1.8^{b}	39.4 ± 2.2^{b}	28.5 ± 1.6^{c}	55.9 ± 1.5^{b}	50.2 ± 1.6^{c}	3.6 ± 0.2^{a}	7.8 ± 0.4^{a}
Extender D	47.1 ± 3.7^{a}	58.7 ± 3.8^{c}	19.1 ± 1.8^{b}	29.1 ± 2.2^{c}	32.8 ± 1.6^{bc}	63.9 ± 1.5^{a}	51.2 ± 1.6^{bc}	3.2 ± 0.2^{ab}	7.4 ± 0.4^{a}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	0.161

Different letters within each column denote statistical differences (P<0.050). Data are shown as the mean ± SEM.

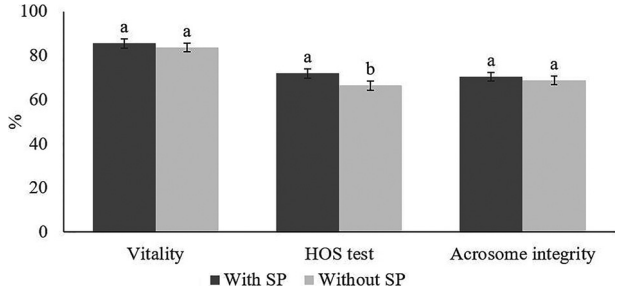

Fig. 1. Effects of SP on the percentage of vitality, HOS test, and acrosome integrity of rabbit spermatozoa from the four extender groups (A–D). Different letters within each diagnostic test denote statistical differences (P<0.050).

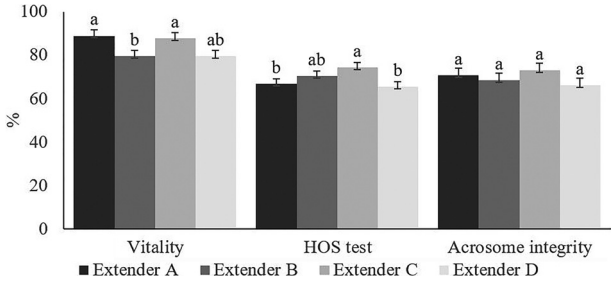

Fig. 2. Percentage of vitality, HOS test, and acrosome integrity of rabbit spermatozoa diluted with extender A, extender B, extender C, or extender D from the with and without SP groups. Data are shown as the mean ± SEM. Different letters within each diagnostic test denote statistical differences (P<0.050).

significant (P<0.050). Even though SP and non-SP samples showed rather similar data for sperm quality (motility, vitality, and acrosome integrity), sperm preserved with SP showed a significanly high percentage (P=0.020) in the HOS test (SP, 71.9 ± 1.6%; non-SP, 66.5 ± 1.6%). On the other hand, non-SP sperm whad slightly higher kinetic parameters related to the velocity and trajectory of the spermatozoa, specifically the LIN (SP, 32.2 ± 1.2%; non-SP, 36.3 ± 1.3%), STR (SP, 60.1 ± 1.1%; non-SP, 62.8 ± 1.1%) and WOB (SP, 53.0 ± 1.2%; non-SP, 56.2 ± 1.2%), showing faster spermatozoa and with further linear trajectories.

Regardless of the presence of SP in the sample, the effect of the extender plays an important role in the quality of rabbit semen preservation according to Table 2 and Fig. 2. All the parameters studied, excepting BCF and acrosome integrity, were affected by the type of extender used (P<0.050). Extender A resulted in the highest values for kinematic parameters related to motility such as VSL (32.9 ± 2.0 μm/s), VAP (49.1 ± 2.5 μm/s), LIN (41.2 ± 1.9%), STR (64.7 ± 1.7%), and WOB (62.1 ± 1.8%). However, other parameters, such as VCL (81.3 ± 3.8 μm/s), ALH (3.6 ± 0.2 μm), and the HOS test (74.3 ± 2.3%), were higher in the samples treated with extender C. On the other hand, both extenders A and C showed great results for MOT (63.1 ± 4.3% and 63.4 ± 3.7%, respectively) and vitality (88.9 ± 2.6% and 87.7 ± 2.7%, respectively). In most cases, samples diluted with extenders B and D showed worse data for sperm quality.

Considering all the sperm parameters studied, the quality of spermatozoa decreased with duration of storage (P=0.025) (Table 3 and Fig. 3). The first significant (P<0.001) drop oin the percentage of motile sperm was observed from 24 (58.4 ± 3.7%) to 48 h (44.1 ± 3.7%).

Table 3. Effects of duration of storage on kinematic parameters from the four extender (A–D) groups and with SP and without SP groups

Duration of storage (h)	MOT (%)	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)	LIN (%)	STR (%)	WOB (%)	ALH (μm)	BCF (Hz)
4	63.5 ± 3.7^{a}	75.9 ± 3.8^{ab}	33.9 ± 1.8^{a}	47.3 ± 2.2^{a}	44.3 ± 1.6^{a}	71.0 ± 1.5^{a}	61.8 ± 1.6^{a}	3.0 ± 0.2^{b}	9.4 ± 0.4^{a}
24	58.4 ± 3.7^{a}	82.4 ± 3.8^{a}	24.2 ± 1.8^{b}	41.2 ± 2.2^{a}	29.5 ± 1.6^{b}	58.5 ± 1.5^{b}	50.1 ± 1.6^{c}	3.6 ± 0.2^{a}	8.4 ± 0.4^{a}
48	44.0 ± 3.7^{b}	66.7 ± 3.8^{b}	18.0 ± 1.8^{c}	31.9 ± 2.2^{b}	28.6 ± 1.6^{b}	56.9 ± 1.5^{b}	49.2 ± 1.6^{c}	3.2 ± 0.2^{ab}	7.0 ± 0.4^{b}
72	27.4 ± 5.0^{c}	47.5 ± 5.1^{c}	14.5 ± 2.4^{c}	25.3 ± 3.0^{b}	34.7 ± 2.2^{b}	58.8 ± 2.0^{b}	58.1 ± 2.1^{b}	2.9 ± 0.2^{b}	3.9 ± 0.5^{c}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.023	<0.001

Means marked with an asterisk within each column denote statistical differences (P<0.050). Data are shown as the mean ± SEM.

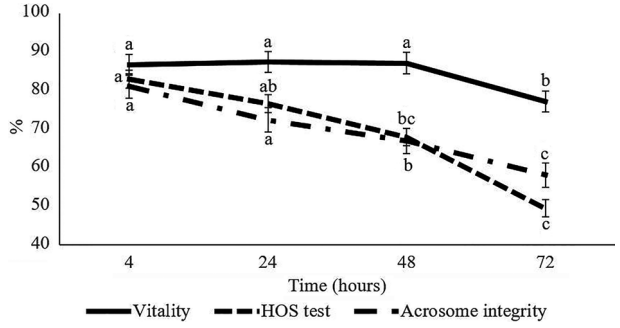

Fig. 3. Percentage of vitality, HOS test, and acrosome integrity of rabbit spermatozoa at 4, 24, 48, and 72 h from the four extender (A–D) groups and with SP and without SP groups. Data are shown as the mean ± SEM. Different letters within each diagnostic test denote statistical differences (P<0.050).

Nonetheless, the sharpest decline in MOT was observed at 72 h of storage (27.4 ± 5.1% motile spermatozoa). Vitality was maintained until 72 h, dropping slightly from 86.6 ± 2.7% live spermatozoa at 4 h to 77.2 ± 2.5% alive spermatozoa after 72 h of storage. Finally, the sperm plasma and acrosome membranes were damaged progressively until 72 h of storage, with 49.5 ± 2.3% and 58.1 ± 3.2% of spermatozoa at that moment having an intact sperm plasma membrane and no damage of the acrosome membrane, respectively. Other parameters related to the kinematics of the spermatozoa, such as VSL, LIN, STR, and WOB, underwent a sharp decline after 24 h of storage. After 48 h of storage, a decrease was observed in the data for the rest of the studied kinematic parameters (VCL, VAP, ALH, and BCF).

Discussion

There are few studies about the effect of SP on rabbit semen preservation. In the present study, even though only significant differences were observed in the HOS test, the percentage of motile spermatozoa, vitality, HOS test, and acrosome integrity values were slightly better when SP was not removed. This observation is in agreement with previous findings, such as those of Castellini *et al*. (2000) [10], who reported the effect of SP with diluents containing decreasing ratios of SP/Tris citrate-glucose and concluded that a sharp decline in motility parameters was observed at dilutions higher than 1/10 and that vitality decreased from 92 ± 6.4% in SP samples to 48 ± 6.4% in samples without SP athat were only diluted with Tris citrate-glucose. Similarly, Aksoy *et al*. (2008) [2] studied the effect of SP removal by simple centrifugation or separation through Percoll and reported that the control group, samples without removal of the SP, had higher percentages of intact sperm head membranes (69.9 ± 2.5%) and intact sperm plasma membranes (39.9 ± 3.5%) than samples centrifuged (33.5 ± 3.1% and 26.0 ± 4.0%, respectively) or separated by Percoll (40.2 ± 3.3% and 25.9 ± 4.5%, respectively). Of note, the present study obtained a higher percentage of intact sperm plasma membranes than Aksoy *et al*. [2] but a similar percentage of intact acrosome membranes in samples diluted with SP. In spite of not having found great differences between samples with SP and samples without SP, all the authors agreed that SP might have a protective effect on rabbit sperm membranes and maintain greater sperm motility. This could be due to the properties of SP. To our knowledge, SP stimulates LH secretion, ovulation, and luteal gland development of induced and spontaneous ovulation species such as rabbits [1]. In addition, the process to remove SP from the samples might induce a considerable amount of stress and damage in the membranes of the spermatozoa, which would explain the decreases in MOT and the parameters which measure the integrity of the sperm plasma and acrosome membranes in non-SP samples.

Indeed, motility is not the only good parameter to analyse; other parameters related to motility such as VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF have

also been found to predict the likelihood of a spermatozoon reaching an oocyte [19, 21, 26]. To date, no kinematic parameters of rabbit spermatozoa have been studied previously by other authors. To our knowledge, the report of Castellini *et al*. [10] is the only one showing data relating kinematic parameters with the effect of SP in rabbit spermatozoa, and it noted that samples with SP had the highest values for the VAP, VCL, VSL, and LIN parameters. Hagen *et al*. [19] observed that samples with SP showed the highest percentage of motile spermatozoa and that non-SP samples diluted with BSA showed greater velocity. Other authors have studied the relationship between kinematic parameters and the *in vitro* fertilization (IVF) rate in human sperm. On one hand, Lui *et al*. (1991) [26] demonstrated that the LIN parameter was the most significant correlate of IVF rate and that VSL was also positively correlated with the IVF rate, but other parameters like ALH and VCL were not significant. On the other hand, a research performed by Hirano *et al*. (2001) [21] on human spermatozoa indicated that kinematic parameters (mostly VCL, VSL, VAP, and STR) provided a reliable estimation of the fertilizing ability of human sperm. In the present research, statistical differences ($P<0.050$) were observed for the LIN, STR, and WOB parameters. Interestingly, in agreement with Hagen *et al*. (2002) [19], these parameters and VCL, VSL, and VAP showed greater sperm movement characteristics in samples without SP. Droplets and vesicles present in rabbit semen have similar sizes to spermatozoa and probably act to modulate the motility characteristics, capacitation process, and acrosome reaction [11, 32, 43]. Once SP is removed, droplets and vesicles in it are also eliminated, and all are replaced by an obstacle-free media. We suggest that the ease of movement in a SP, droplets, and vesicles-free media makes sperm show faster velocities and further linear trajectory parameters. Moreover, if we consider that LIN, VSL, VAP, VCL, and STR could be used to predict the fertilizing ability of the spermatozoa [21, 26], non-SP samples would be the most suitable for artificial insemination.

The extender plays an important role in seminal preservation; it should provide nutrients to sperm and protect it against cold shock. Unlike in other domestic or wild animals [7, 12, 15, 27, 30, 34], glycerol has not been the cryoprotectant of first choice for use in rabbit semen preservation due to its toxicity [13]. It is supposed that the toxic effect of glycerol on rabbit sperm is due to its low water permeability coefficient and a high activation energy [13], triggering the denaturation of proteins, osmotic stress, alteration of actin interactions, and induction of protein-free membrane blisters [16, 23, 35]. Therefore, cryoprotectants with lower molecular weights and high permeability such amide solvents (DMF or NMP) could be suitable for use in rabbits. In this study on rabbit sperm preservation, it was observed that the DMF cryoprotectant maintained sperm quality better than glycerol, as in stallion sperm preservation [36, 37]. Furthermore, samples diluted with INRA and INRA plus DMF showed the highest percentages of motile spermatozoa and vitality; however, addition of DMF to INRA diluent had a greater protective effect on the sperm plasma membrane against the damage caused by the semen preservation procedure. Additionally, INRA showed better results for kinematics parameters such as VSL, VAP, LIN, STR, and WOD. Glycerol or NMP could not be used to replace the DMF cryoprotectant because of the poor results obtained. This demonstrated that addition of the DMF cryoprotectant to the INRA diluent has a protective effect on the membranes of spermatozoa, improving semen quality during rabbit sperm preservation. Likewise, we support the hypothesis regarding the toxicity of glycerol as a cryoprotectant in rabbit sperm preservation [3, 16, 35].

As other authors have published, the quality of spermatozoa decreased with the duration of storage [28, 39] due to the peroxidation of spermatozoa during conservation [10]. López and Alvariño (1998) [28] demonstrated that fertility also decreased when does were inseminated with samples stored for 72 h (67.6% of fertility); however, in does inseminated with samples stored for 2 or 24 h after collection, the fertility obtained was around 84%. Furthermore, Roca *et al*. (2000) [39] reported that the percentage of motile spermatozoa in semen stored over 96 h decreased from around 80% to 60–20% depending on the diluent used. As expected, in this study the sperm quality of all the samples decreased throughout storage, as other authors previously reported, showing a considerable drop after 48 h in all parameters studied.

In conclusion, both motility and kinematic parameters are useful for estimating the quality of spermatozoa during the preservation process. SP might have a protective effect on rabbit sperm membranes and maintain better sperm motility. Removal of SP from the samples in the present study required a centrifugation process which

might induce a considerable amount of stress and damage in the membranes of spermatozoa. Nevertheless, removal of SP showed a beneficial effect on kinematic parameters, suggesting that SP could affect the linear movement of spermatozoa. There were significant differences between the extenders used. The addition of glycerol and NMP cryoprotectants to the INRA diluent does not improve rabbit sperm quality. Nevertheless, the DMF cryoprotectant exerts a protective effect on the membranes of spermatozoa, improving semen quality during rabbit sperm preservation at 16°C.

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